

Camptothecins with a modified lactone ring

The invention described herein relates to compounds useful as medicaments, particularly derivatives of camptothecin with structural modifications of the lactone ring, to processes for their preparation, to their use as active agents endowed with topoisomerase I inhibiting activity and to pharmaceutical compositions containing them as active ingredients.

Background to the Invention

Camptothecin is an alkaloid isolated by Wall *et al.* (*J. Am. Chem. Soc.*, 88, 3888-3890 (1966)) for the first time from the tree *Camptotheca acuminata*, a plant native to China, belonging to the *Nyssaceae* family. The molecule consists of a pentacyclic structure with a lactone in the E ring, which is essential for cytotoxicity.

For a review of the camptothecins and the problems relating to their use as medicaments, as well as the resolution of a number of such problems, see European Patent EP 1044977, filed in the name of the applicant.

As regards the problem of the lactone ring, which is a portion of the molecule essential for the camptothecins to be able to exercise their pharmacological activity, one aspect which has yet to be fully resolved is the stability of the ring itself, which, in turn, is responsible for the half-life of the drug.

Patent application WO 97/00876, filed in the name of Societ  de Conseils de Recherches et d'Applications Scientifiques, and published on 09.01.1997, describes camptothecins in which the lactone ring has been modified from its original α -hydroxylactone structure to a β -hydroxylactone structure (homocamptothecins), bringing the lactone cycle up from six to seven members. These compounds inhibit topoisomerase I DNA relaxation activity and are endowed with cytotoxic

activity against several tumour lines. The β -hydroxylactone structure is defined as a lactone that involves the presence of a supplementary carbon atom between the carboxyl carbon atom and the carbon atom in α - bearing the hydroxy in the α -hydroxylactone structure. To increase the stability of the lactone ring, the inventors suggest substituents on the supplementary carbon atom, and the substituents indicated are the lower alkyls together with the lower alkoxy, halogen or hydroxy. In the patent application mentioned no evidence of improved stability of the lactone ring is provided. In a subsequent patent application, WO 98/28304, published on 02.07.1998, the same applicant describes further camptothecins with a β -hydroxylactone structure, where the hydroxy group is functionalised with groups that are capable of restoring it *in vivo*, thus effectively furnishing prodrugs of the molecules described in the preceding patent application, and also resolving the problem of the severe side effects of products in the present state of the art. In this case, too, no experimental evidence is provided that the technical problem has been solved. In *J. Med. Chem.*, 1998, Vol 41, No 27, 5410-5419, the same inventors as in the abovementioned patent applications indicate the lactone in position 7, therein described, as an instrument for increasing the stability of the lactone ring, and thus as a useful model for elaborating further camptothecin derivatives. See also *Bioorg. Med. Chem. Lett.*, 9, (1999) 2599-2602; *Biochemistry*, 1999, 38, 15556-15563; *Cancer Research*, 59 2939-2943. Other modifications of homocamptothecin on the A and B rings are described in WO 00/61146, University of Pittsburgh et al., published on 19.10.2000, and in *J. Med. Chem.*, 1999, 42, 3018-3022 for the so-called "homosilatecans", which are potent, stable topoisomerase I inhibitors. Homocamptothecins with further modifications are described in *J. Med. Chem.*, 2000, 43, 2285-2289, *Anti-cancer Drug Design*, (2001), 12, 9-19, where the anticancer activity is increased thanks to the fluoridation of the A ring. See also *Anti-cancer Drug Design*, (2001), 16, 27-36, for the substitution with chlorine in position 12.

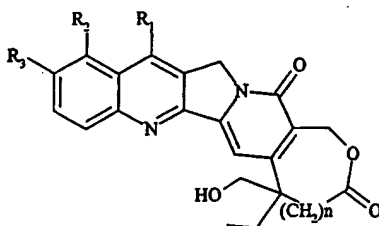
The problem of the hydrosolubility of the homocamptothecins is addressed in US 6,291,676, University of Kentucky, published on 18.09.2001 with various substitutions of the (poly)alkylamine type in position 7.

However much in the design of new drugs various problems are encountered of a physicochemical nature, such as the stability of the molecule in plasma or its hydrosolubility for formulatory purposes, there is a constant search for a better therapeutic index.

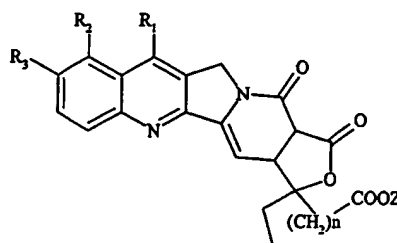
Summary of the Invention

It has now surprisingly been found that substituted 7-oxime camptothecins, with a modified lactone ring, are endowed with substantial anticancer activity and are stable in plasma. These compounds have a better therapeutic index.

The objects of the invention described herein are therefore compounds of general formula (I) and (II):



(I)



(II)

where:

R₁ is hydrogen or a -C(R₅)=N-O-R₄ group, in which R₄ is hydrogen or a straight or branched C₁-C₅ alkyl or C₁-C₅ alkenyl group, or a C₃-C₁₀ cycloalkyl group, or a straight or branched (C₃-C₁₀) cycloalkyl - (C₁-C₅) alkyl group, or a C₆-C₁₄ aryl group, or a straight or branched (C₆-C₁₄) aryl - (C₁-C₅) alkyl group, or a heterocyclic group or a straight or branched heterocyclo - (C₁-C₅) alkyl group, said heterocyclic group

containing at least one heteroatom selected from an atom of nitrogen, optionally substituted with an (C₁-C₅) alkyl group, and/or an atom of oxygen and/or of sulphur; said alkyl, alkenyl, cycloalkyl, cycloalkyl-alkyl, aryl, aryl-alkyl, heterocyclic or heterocyclo - alkyl groups can optionally be substituted with one or more groups selected from the group consisting of: halogen, hydroxy, C₁-C₅ alkyl, C₁-C₅ alkoxy, phenyl, cyano, nitro, and -NR₆R₇, where R₆ and R₇, which may be the same or different, are hydrogen, straight or branched (C₁-C₅) alkyl, the -COOH group or one of its pharmaceutically acceptable esters; or the -CONR₈R₉ group, where R₈ and R₉, which may be the same or different, are hydrogen, straight or branched (C₁-C₅) alkyl; or

R₄ is a (C₆-C₁₀) aroyl or (C₆-C₁₀) arylsulphonyl residue, optionally substituted with one or more groups selected from: halogen, hydroxy, straight or branched C₁-C₅ alkyl, straight or branched C₁-C₅ alkoxy, phenyl, cyano, nitro, -NR₁₀R₁₁, where R₁₀ and R₁₁, which may be the same or different, are hydrogen, straight or branched C₁-C₅ alkyl; or:
R₄ is a polyaminoalkyl residue; or

R₄ is a glycosyl residue;

R₅ is hydrogen, straight or branched C₁-C₅ alkyl, straight or branched C₁-C₅ alkenyl, C₃-C₁₀ cycloalkyl, straight or branched (C₃-C₁₀) cycloalkyl - (C₁-C₅) alkyl, C₆-C₁₄ aryl, straight or branched (C₆-C₁₄) aryl - (C₁-C₅) alkyl;

R₂ and R₃, which may be the same or different, are hydrogen, hydroxy, straight or branched C₁-C₅ alkoxy;

n = 1 or 2,

Z is selected from hydrogen, straight or branched C₁-C₄ alkyl;

the N₁-oxides, the racemic mixtures, their individual enantiomers, their individual diastereoisomers, their mixtures, and their

pharmaceutically acceptable salts, with the proviso that, in formula (I), R_1 , R_2 and R_3 cannot be simultaneously hydrogen.

The present invention includes the use of compounds of the above-mentioned formulae (I) and (II) as active ingredients for medicaments, particularly for medicaments which are useful as topoisomerase I inhibitors. Among the therapeutic applications deriving from topoisomerase I inhibition we should mention the treatment of tumours and parasitic or viral infections.

The present invention includes pharmaceutical compositions containing compounds of formula (I) and/or formula (II) as active ingredients, in admixture with pharmaceutically acceptable vehicles and excipients.

The present invention also includes the processes for the preparation of compounds of formula (I) and (II), and the key intermediate products.

Detailed description of the invention

Within the framework of the present invention, examples of the straight or branched C_1 - C_5 alkyl group, are understood to include methyl, ethyl, propyl, butyl, pentyl and their possible isomers, such as, for example, isopropyl, isobutyl, and ter-butyl.

Examples of the branched or straight C_1 - C_5 alkenyl group are methyldiene, ethyldiene, vinyl, allyl, propargyl, butylene, and pentylene, where the double carbon-carbon bond may be situated in the various possible positions of the alkylene ring, which can also be branched in the context of the isomery allowed.

Examples of the C_3 - C_{10} cycloalkyl group are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl, and polycyclic groups, such as, for example, adamantyl.

Examples of the straight or branched (C₃-C₁₀) cycloalkyl - (C₁-C₅) alkyl group are cyclopropylmethyl, 2-cyclopropylethyl, 1-cyclopropylethyl, 3-cyclopropylpropyl, 2-cyclopropylpropyl, 1-cyclopropylpropyl, cyclobutylmethyl, 2-cyclobutylethyl, 1-cyclobutylethyl, 3-cyclobutylpropyl, 2-cyclobutylpropyl, 1-cyclobutylpropyl, cyclohexylmethyl, 2-cyclohexylethyl, 1-cyclohexylethyl, 3-cyclohexylpropyl, 2-cyclohexylpropyl, 1-cyclohexylpropyl, 5-cyclohexylpentyl, 3-cyclohexylpentyl, 3-methyl-2-cyclohexylbutyl, 1-adamantylethyl, 2-adamantylethyl, adamantylmethyl.

Examples of the straight or branched (C₆-C₁₄) aryl or (C₆-C₁₄) aryl - (C₁-C₅) alkyl group are phenyl, 1- or 2-naphthyl, anthracenyl, benzyl, 2-phenylethyl, 1-phenylethyl, 3-phenylpropyl, 2-anthracenylpropyl, 1-anthracenylpropyl, naphthylmethyl, 2-naphthylethyl, 1-naphthylethyl, 3-naphthylpropyl, 2-naphthylpropyl, 1-naphthylpropyl, cyclohexylmethyl, 5-phenylpentyl, 3-phenylpentyl, 3-methyl-2-phenylbutyl.

Examples of the straight or branched heterocyclic or heterocyclo - (C₁-C₅) alkyl group are thienyl, quinolyl, pyridyl, N-methylpiperidiny, 5-tetrazolyl, 2-(4,5-dihydroxazolyl), 1,2,4-oxadiazolidin-3-yl-5-one, purine and pyrimidine bases, e.g. uracyl, optionally substituted as indicated in the general definitions above.

Examples of the (C₆-C₁₀) aroyl groups are benzoyl and naphthoyl.

Examples of the (C₆-C₁₀) arylsulphonyl groups are tosyl and benzoylsulphonyl.

What is meant by halogen is fluorine, chlorine, bromine and iodine.

Examples of substituted groups are pentafluorophenyl, 4-phenylbenzyl, 2,4-difluorobenzyl, 4-aminobutyl, 4-hydroxybutyl, dimethylaminoethyl, p-nitrobenzoyl, p-cyanobenzoyl.

An example of the polyaminoalkyl residue is $-(CH_2)_m-NR_{12}-(CH_2)_p-NR_{13}-(CH_2)_q-NH_2$, where m, p and q are whole numbers from 2 to 6 inclusive and R_{12} and R_{13} are a straight or branched (C_1 - C_5) alkyl group, for example 4-aminobutyl-2-aminoethyl, 3-amino-propyl-4-aminobutyl, 3-aminopropyl-4-aminobutyl-3-aminopropyl.

Examples of the glycosyl residue are 6-D-galactosyl and 6-D-glucosyl.

Examples of pharmaceutically acceptable salts are, in the case of atoms of nitrogen of a basic nature, salts with pharmaceutically acceptable acids, both inorganic and organic, such as, for example, hydrochloric acid, sulphuric acid, acetic acid, or, in the case of an acid group, such as carboxyl, salts with pharmaceutically acceptable bases, such as, for example, alkaline and alkaline-earth hydroxides, ammonium hydroxide, and amines, including heterocyclic amines.

One first group of preferred compounds comprises formula (I) compounds in which the lactone ring is 7- or 8-membered, particularly 7-membered.

A second group of preferred compounds comprises formula (II) compounds in which the lactone ring is 5-membered.

In the context of the above-mentioned two preferred groups, those preferred are the formula (I) compounds, in which R_4 is different from hydrogen, and particularly a straight or branched C_1 - C_5 alkyl or C_1 - C_5 alkenyl or C_3 - C_{10} cycloalkyl, or (C_3 - C_{10}) cycloalkyl - (C_1 - C_5) alkyl group, or a straight or branched C_6 - C_{14} aryl, or (C_6 - C_{14}) aryl - (C_1 - C_5) alkyl group, or a straight or branched heterocyclic or heterocyclo - (C_1 - C_5) alkyl group, said heterocyclic group containing at least one heteroatom selected from an atom of nitrogen, optionally substituted with a (C_1 - C_5) alkyl group, and/or of oxygen and/or of sulphur; said alkyl, alkenyl, cycloalkyl, aryl, aryl-alkyl, heterocycle or heterocyclo-alkyl groups, may be substituted with one or more groups selected from: halogen, hydroxy, C_1 - C_5 alkyl, C_1 - C_5 alkoxy, phenyl, cyano, nitro, $-NR_6R_7$, where

R₆ and R₇, which may be the same or different, are straight or branched (C₁-C₅) alkyl; the -COOH group or one of its pharmaceutically acceptable esters; or the -CONR₈R₉ group, where R₈ and R₉, which may be the same or different, are hydrogen, straight or branched (C₁-C₅) alkyl, according to the definitions outlined above as examples.

An initial group of particularly preferred compounds consists of formula (I) compounds, with a 7-membered lactone ring, and, among these, particularly:

R,S-7-methoxyiminomethyl-homocamptothecin;
R,S-7-ethoxyiminomethyl-homocamptothecin;
R,S-7-isopropoxyiminomethyl-homocamptothecin;
R,S-7-(2-methylbutoxy)iminomethyl-homocamptothecin;
R,S-7-(1-t-butoxy)iminomethyl-homocamptothecin (ST2127);
R,S-7-(4-hydroxybutoxy)iminomethyl-homocamptothecin;
R,S-7- triphenylmethoxyiminomethyl-homocamptothecin;
R,S-7-carboxymethoxyiminomethyl-homocamptothecin;
R,S-7-aminoethoxyiminomethyl-homocamptothecin;
R,S-7-(N,N-dimethylaminoethoxy)iminomethyl-homo-camptothecin;
R,S-7-allyloxyiminomethyl-homocamptothecin;
R,S-7-cyclohexyloxyiminomethyl-homocamptothecin;
R,S-7-cyclohexylmethoxyiminomethyl-homocamptothecin;
R,S-7-cyclooctyloxyiminomethyl-homocamptothecin;
R,S-7-cyclooctylmethoxyiminomethyl-homocamptothecin;
R,S-7-benzyloxyiminomethyl-homocamptothecin (ST2143);
R,S-7-(benzyloxy)iminophenylmethyl-homocamptothecin;
R,S-7-(1-benzyloxy)iminoethyl-homocamptothecin;
R,S-7-(1-t-butoxy)iminoethyl-homocamptothecin;
R,S-7-p-nitrobenzyloxyiminomethyl-homocamptothecin;
R,S-7-p-methylbenzyloxyiminomethyl-homocamptothecin;
R,S-7-pentafluorobenzyloxyiminomethyl-homocamptothecin;
R,S-7-p-phenylbenzyloxyiminomethyl-homocamptothecin;
R,S-7-(2,4-difluorobenzylmethoxy)iminomethyl-homocamptothecin;
R,S-7-(4-t-butylphenylmethoxy)iminomethyl-homocamptothecin;

R,S-7-(1-adamantyloxy)iminomethyl-homocamptothecin;
R,S-7-(1-adamantylmethoxy)iminomethyl-homocamptothecin;
R,S-7-(2-naphthalenyloxy)iminomethyl-homocamptothecin;
R,S-7-(9-anthracenylmethoxy)iminomethyl-homo-camptothecin;
R,S-7-(6-uracyl)methoxyiminomethyl-homocamptothecin;
R,S-7-(4-pyridil)methoxyiminomethyl-homocamptothecin;
R,S-7-(2-thienyl)methoxyiminomethyl-homocamptothecin;
R,S-7-[(N-methyl)-3-piperidiny]methoxyiminomethyl-
homocamptothecin;
R,S-7-hydroxyiminophenylmethyl-homocamptothecin.

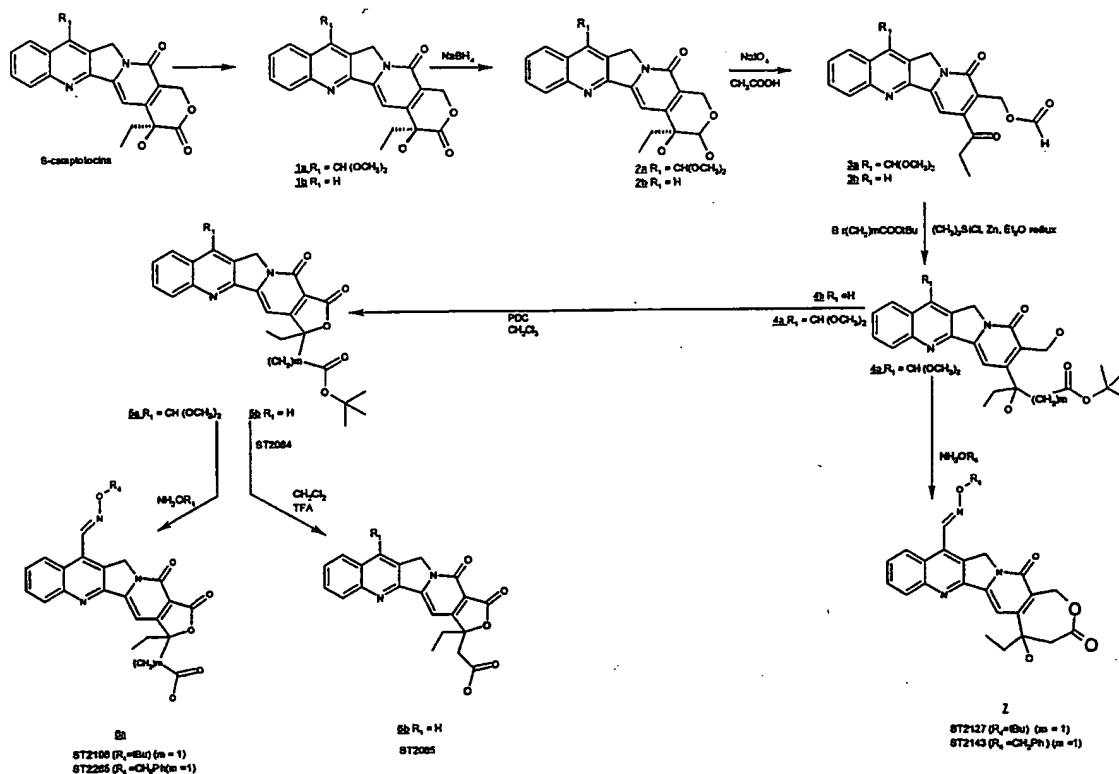
Among these compounds, those most preferred are R,S-7-(1-t-butoxy)iminomethyl-homocamptothecin (ST2127) and R,S-7-benzyl-oxyiminomethyl-homocamptothecin (ST2143).

A second group of particularly preferred compounds consists of formula (II) compounds, with a 5-membered lactone ring and with the same meanings of R₁ as in the preceding group.

Among these compounds, those which are most preferred are {10-[(E)-(ter-butoxyimino)methyl]-3-ethyl-1,13-dioxo-11,13-dihydro-1H,3H-furo[3',4':6,7]indolizino[1,2-b]quinolin-3-yl}acetic acid (ST2196), (10-{(E)-[(benzyloxy)imino]methyl}-3-ethyl-1,13-dioxo-11,13-dihydro-1H,3H-furo[3',4':6,7]indolizino[1,2-b]quinolin-3-yl)acetic acid (ST2285) and (3-ethyl-1,13-dioxo-11,13-dihydro-1H,3H-furo[3',4':6,7]indolizino[1,2-b]quinolin-3-yl)acetic acid (ST2085).

In a first preferred embodiment of the invention, compounds of general formula (I) are envisaged, where the lactone ring is 7- or 8-membered. In a second preferred embodiment of the invention, compounds of general formula (II) are envisaged, where the lactone ring is 5-membered.

The formula (I) compounds can be prepared with the process described here below and exemplified for the preferred compounds according to the present invention.



It is quite obvious to the person with ordinary experience in the field that the process scheme applies to all the compounds covered by formulae (I) and (II), since the method for obtaining the two classes of compounds with the 7-/8-membered lactone ring, on the one hand, and the 5-membered ring, on the other, is fully described. The preparation of the various compounds covered by the two formulae differs in the substituent in position 7.

This preparation is described amply and completely in the above-mentioned EP 104977.

As regards the formula (I) and (II) compounds in which R₁ is hydrogen, the starting compound is camptothecin, optionally substituted in

positions 8 and 9 according to the meanings of the R_2 and R_3 groups envisaged above.

In the case of formula (I) and (II) compounds in which R_1 is other than hydrogen, the functionalisation of position 7 to obtain the final compound cannot happen before the modification of the original lactone ring of camptothecin, both in the sense of its amplification to 7 or 8 members and in the sense of its restriction to 5 members. For this purpose, it has proved necessary to find a suitable intermediate product for the synthesis pathway envisaged. This key intermediate is 7-(dialkoxymethyl)camptothecin. This new compound is an additional object of the present invention. Among these, the preferred compound is 7-(dimethoxymethyl)camptothecin. The camptothecin is reacted with the desired alcohol, which can also be used as a reaction medium, in the presence of a mineral acid, such as, for example, sulphuric acid, and a suitable oxidising system, such as iron sulphate/hydrogen peroxide, then a further oxidising agent, such as manganese dioxide to obtain 7-(dialkoxymethyl)camptothecin.

Camptothecin, or its 7-(dialkoxymethyl)-derivative, are subjected to selective reduction of the carbonyl in position 19, to obtain the corresponding 19,20-dihydroxy derivative. The reduction is carried out in the presence of a reducing agent, for example, mixed hydrides of Al or B and exemplified in the scheme by sodium borohydride, from 1 to 10 equivalents in the presence of an alcoholic solvent for a period of time ranging from 1 to 16 h at a temperature ranging from room temperature to 50°C. The solvent is subsequently evaporated and the crude product is used in the subsequent step, where the E ring, in the form of the 19,20-dihydroxy derivative, is subjected to opening with from 1 to 10 equivalents of an oxidising agent, such as, for example, periodate or lead acetate. The reaction is conveniently carried out in an organic solvent, such as, for example, toluene, methylene chloride or acetic acid, for a time period ranging from 1 to 24 h, at a temperature ranging from room temperature to 50°C. The solvent is removed in vacuo and the product is finally purified by

chromatography or some other equivalent means. The intermediate product thus obtained is in turn dissolved in a suitable solvent medium, preferably a mixture of solvents, and then subjected to the well known Reformatsky reaction, in which the ω -bromocarboxylic acid is suitably selected as a function of the n value envisaged in formula (I) or (II). At this point, in the context of the embodiment of the present invention relating to formula (I) compounds, to the product of the Reformatsky reaction, dissolved in a suitable mixture of solvents, such as, for example, methylene chloride, acetic acid, and dimethyl formamide, optionally in the presence of an acid (for example, trifluoroacetic acid or a Lewis acid) and of a condensing agent (dicyclohexylcarbodiimide - DCC - or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) or a dehydrating agent (for example, sodium or magnesium sulphate, or molecular sieves), are added from 1 to 10 equivalents of a suitable hydroxylamine NH_2OR_4 , also in the form of a salt, where R_4 has the meanings described above for times ranging from 1 to 24 h, at a temperature ranging from room temperature to the boiling point of the solvent, to obtain the final formula (I) compound. The final product is isolated by removal of the solvent and final purification, for example, by chromatography. Alternatively, in the context of the second preferred embodiment of the invention, that is to say of formula (II) compounds in which the lactone ring is 5-membered, the product of the Reformatsky reaction, after being dissolved in a mixture of organic solvents, such as methylene chloride, acetic acid, and acetonitrile, is treated with from 1 to 10 equivalents of an oxidising agent (for example, chromic acid, pyridinium dichromate - PDC - manganese oxide, Na_2RuO_4) at a temperature ranging from 0°C to the boiling point of the solvent, for a time period ranging from 30 minutes to 24 h. The solvent is then removed in vacuo and the product purified by chromatography. The resulting compound is dissolved in a suitable organic solvent, such as, for example, methylene chloride) or an aqueous solvent and subjected to acid hydrolysis with an organic or inorganic acid, such as trifluoroacetic acid, hydrochloric acid, or perchloric acid) for a time period ranging from 1 to 24 h at a temperature ranging from 0°C to the

boiling point of the solvent. The latter is then removed and the product isolated by crystallisation. If desired, the product is finally reacted with the hydroxylamine NH_2OR_4 as seen above. If formula (II) compounds, where Z is hydrogen, are desired, the compound obtained by the process described here above will be subjected to suitable treatment to release the carboxylic function according to conventional ester hydrolysis methods with which the experts in the field are fully familiar.

The reaction with the hydroxylamine NH_2OR_4 is amply described in the above-mentioned patent EP 1044977, as is the preparation of possible N-oxides.

Pharmaceutically acceptable salts are obtained with conventional methods reported in the literature and do not require any further description.

The compounds described in the present invention are topoisomerase I inhibitors and therefore are useful as medicaments, particularly for the treatment of diseases that benefit from the inhibition of said topoisomerase. In particular, the compounds according to the present invention display antiproliferative activity and are therefore used on account of their therapeutic activity and possess physicochemical properties that make them suitable for formulation in pharmaceutical compositions.

The pharmaceutical compositions contain at least one formula (I) and/or formula (II) compound as an active ingredient, in an amount such as to produce a significant therapeutic effect. The compositions covered by the present invention are wholly conventional and are obtained with methods which are common practice in the pharmaceutical industry. According to the administration route opted for, the compositions will be in solid or liquid form, suitable for oral, parenteral, or intravenous administration. The compositions according to the present invention contain, along with the active ingredient, at

least one pharmaceutically acceptable vehicle or excipient. Particularly useful may be formulation coadjuvants, such as, for example, solubilisers, dispersant agents, suspension agents and emulsifiers.

The formula (I) compounds can also be used in combination with other active ingredients, such as, for example, other anticancer drugs or other drugs with antiparasitic or antiviral activity, both in separate and in single dosage forms.

The compounds according to the present invention are useful as medicaments with anticancer activity, for example, in lung cancers, such as non-microcytoma lung cancer, or in colorectal or prostate tumours or gliomas.

The cytotoxic activity of the compounds according to the present invention has been assayed in cell systems of human tumour cells, using the antiproliferative activity test as the method of evaluating the cytotoxic potential.

The cell line used is a non-microcytoma pulmonary adenocarcinoma called NCI H460, belonging to the NSCLC (non small cell lung cancer) class.

Anticancer activity

To evaluate the effect of the compounds according to the present invention, their cytotoxicity against the non-microcytoma lung cancer cell line (NCI-H460) was evaluated. Cells from the American Type Culture Collection (ATCC) were maintained in culture in RPMI 1640 (GIBCO) containing 10% foetal calf serum and gentamicin sulphate at a concentration of 50 µg/ml.

The cells were seeded in a volume of 250 µl in 96-well plates and incubated for 24 h at 37°C. The next day the study compounds were added at scalar concentrations from 1 µM to 0.004 µM, and the cells

were incubated for another 2 h at 37°C in a humidified atmosphere containing 5% CO₂. The cells were washed 3 times, overturning the plates each time and adding PBS. 200 µl/well of RPMI 1640 medium containing 10% FCS were added and the plates were incubated at 37°C for a further 72 h. On day 5, the growth medium was removed by overturning the plates, and 200 µl/well of PBS and 50 µl of 80% cold TCA were added. The plates were then incubated in ice for at least 1 h. The TCA was removed by overturning; the plates were washed 3 times by immersion in distilled water and dried first on blotting paper and then under a hot air jet. 200 µl of 0.4% sulforodamine B in 1% acetic acid were added to all wells. The plates were incubated at room temperature for a further 30 minutes. The sulforodamine B was removed by overturning; the plates were washed by immersion 3 times in 1% acetic acid and then dried first on blotting paper and then with a jet of hot air. 200 µl of Tris base 10 mM were added to all wells and the plates were subjected to stirring for at least 20 minutes. The optical density was measured using a Multiskan spectrophotometer at 540 nm.

Table 1 presents the IC₅₀ values, that is to say the concentration capable of inhibiting 50% of cell survival, for each compound examined, processed using ALLFIT software.

Table 1

Product	NCI-H460 IC ₅₀ (µM)
ST2084	>1
ST2085	>1
ST2127	0.026
ST2143	0.007
ST2196	>1
ST2285	>1

The following examples further illustrate the invention, referring to the scheme indicated above.

Preparation 1

Synthesis of 7-(dimethoxymethyl)camptothecin (ST2337)

To a suspension of 1.53 g (4.4 mmol) of camptothecin in 92 ml of methanol, cooled with an ice bath under stirring, were added slowly 9.2 ml of H₂SO₄ 96%, keeping the temperature of the mixture below 50°C. The suspension thus obtained was heated to reflux temperature; on reaching 50°C, 46 mg of FeSO₄ · 7·H₂O were added and then 3 ml of H₂O₂ 30% dropwise, keeping the reaction at reflux temperature. The reaction was stirred for 2 hours, checking for disappearance of the starting product by TLC. On completion of the reaction, the suspension was cooled to 25°C and 2.8 ml of MnO₂ were added; the mixture was stirred for 2 hours, checking for disappearance of the intermediate product by TLC. The suspension was then filtered through a layer of Celite placed on a Gooch filter. The filtered solution was concentrated to 25 ml and then poured into a solution of NaHCO₃ in water so as to obtain a solution at pH 6. The precipitate was filtered and purified on a silica gel chromatography column (eluents: CH₂Cl₂/MeOH 99/1). 1.02 g (2.42 mmol, 55%) of product were obtained as a yellow solid.

C₂₃H₂₂N₂O₆ (422,4); m.p. (decomp.) = 201°C;

R_f = 0.5 (CH₂Cl₂/MeOH 92/8).

MS (IS): [M+Na]⁺ = 445; [M-1]⁻ = 421.

Elemental analysis: calculated: C 65.40, H 5.21, N 6.64; found: C 65.37, H 5.22, N 6.67.

¹H NMR (CDCl₃) δ = 1.00-1.06 (t, 3H, CH₃), 1.82-1.97 (m, 2H, CH₂), 3.40 (s, 3H, CH₃), 3.43 (s, 3H, CH₃), 5.28-(5.33-5.72)-5.78 (dd, 2H, CH₂), 5.47 (s, 2H, CH₂), 6.24 (s, 1H, CH), 7.62-7.70 (m, 2H, CH + CH_{Ar}), 7.78-7.84 (t, 1H, CH_{Ar}), 8.23-8.33 (m, 2H, CH_{Ar}).

¹³C NMR (CDCl₃) δ = 8.0; 31.9; 52.1; 52.9; 53.2; 66.7; 72.9; 98.1; 100.5; 119.0; 124.6; 125.9; 127.8; 128.4; 130.5; 138.4; 146.2; 149.4; 150.2; 152.7; 158.0; 174.1.

Preparation 2

Synthesis of intermediate product 2a

To a solution of 2.12 g (56 mmol, 3.3 eq) of NaBH_4 in 70 mL of MeOH were added 7.2 g (17 mmol) of 7-dimethyl-acetal camptothecin (**1a**); the mixture thus obtained was stirred at room temperature for 1 h. At the end of this operation acetone was added to destroy the NaBH_4 in excess and the solution was brought to dryness. The crude reaction product was purified by flash chromatography on silica gel (eluent gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 92/8 $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 7/3) to yield 3.7 g (8.7 mmol, 51%) of product as a yellow solid.

$\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_6$ (424.5); R_f = 0.41 (1st isomer), 0.35 (2nd isomer) ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 92/8).

MS (IS): $[\text{MH}]^+ = 425$; $[\text{M}+\text{Na}]^+ = 447$; $[\text{M}-1]^- = 423$.

Elemental analysis: calculated: C 65.09, H 5.66, N 6.60; found C 65.12, H 5.68, N 6.57.

^1H NMR ($\text{DMSO}-d_6$) δ = 0.84-0.90 (t, 3H, CH_3), 1.65-1.73 (m, 2H, CH_2), 3.38 (s, 6H, CH_3), 4.43-(4.50-4.57) 4.64 (dd, 2H, CH_2), 4.98 (s, 1H, CH), 5.28 (s, 2H, CH_2), 6.32 (s, 1H, CH), 7.38 (s, 1H, CH), 7.66-7.73 (t, 1H, CH_{Ar}), 7.8-7.88 (t, 1H, CH_{Ar}), 8.14-8.17 (d, 1H, CH_{Ar}), 8.3-8.33 (d, 1H, CH_{Ar}).

^{13}C NMR ($\text{DMSO}-d_6$) δ = 7.6; 32.4; 50.7; 53.1; 53.5; 58.2; 70.1; 78.3; 92.5; 96.0; 98.4; 100.3; 123.1; 124.9; 127.3; 129.4; 129.9; 137.6; 142.3; 148.3; 150.1; 153.1; 157.1.

Preparation 3

Synthesis of intermediate product 3a

To a solution of 5.52 g (13 mmol) of **2a** in 100 ml of CH_3COOH were added 4.17 g of NaIO_4 (19.5 mmol, 1.5 eq.). The mixture was stirred at room temperature for 16 h; at the end of this operation, the solution was concentrated and diluted with CH_2Cl_2 , then extracted with

NaHCO₃ to neutral pH. The organic phase was dried on Na₂SO₄ and evaporated to dryness. Purification was performed by flash chromatography on silica gel (eluents: CH₂Cl₂/MeOH 98/2). 3.58 g (8.48 mmol, 65%) of product were obtained as a yellow solid.

C₂₃H₂₂N₂O₆ (422.4); m.p. (decomp.) = 150°C;

R_f = 0.6 (CH₂Cl₂/MeOH 95/5).

Elemental analysis: calculated: C 65.40, H 5.21, N 6.64; found C 65.39, H 5.23, N 6.61.

MS (IS): [MH]⁺ = 423; [M+Na]⁺ = 445.

¹H NMR (DMSO-d₆) δ = 1.07-1.2 (t, 3H, CH₃), 2.96-3.3 (m, 2H, CH₂), 3.37 (s, 6H, CH₃), 5.12 (s, 2H, CH₂), 5.18 (s, 2H, CH₂), 6.37 (s, 1H, CH), 7.38 (s, 1H, CH), 7.73-7.79 (t, 1H, CH_{Ar}), 7.86-7.92 (t, 1H, CH_{Ar}), 8.16-8.20 (d, 1H, CH_{Ar}), 8.27 (s, 1H, CH), 8.33-8.37 (d, 1H, CH_{Ar}).

Preparation 4

Synthesis of intermediate product 4a

A suspension of 7.6 g (116 mmol) of zinc in 60 ml of anhydrous (distilled) Et₂O, maintained under argon and under stirring, was activated by dropwise addition of 0.87 ml (6.8 mmol) of chlorotrimethylsilane. The suspension was stirred for 15 minutes, and then brought to reflux temperature. After removing the oil bath, 17.5 ml (118 mmol) of tert-butylbromoacetate were added dropwise at a rate such as to maintain the mixture at reflux temperature: a colourless solution was obtained. After resuming heating, the reaction was maintained at reflux temperature for 1 h; at the end of this period, a suspension of 2.3 g (5.45 mmol) of 3a in 45 ml of anhydrous (distilled) THF was added, keeping the reaction under argon. The mixture thus obtained was stirred at reflux temperature. After 1 h the mixture, which had become a yellow solution, was spent with 200 ml of saturated ammonium chloride solution and extracted with CH₂Cl₂; the organic phase was dried on Na₂SO₄, the solvent was evaporated and the crude product purified by flash chromatography on silica gel (eluent gradient CH₂Cl₂ - CH₂Cl₂/MeOH 98/2). 1.6 g (3.14 mmol, 58%)

of product were obtained as a yellow solid.

$C_{28}H_{34}N_2O_7$ (510.6); m.p. (decomp.) = 190°C;

R_f = 0.3 (CH_2Cl_2 /MeOH 98/2); R_f = 0.5 (CH_2Cl_2 /MeOH 95/5).

MS (IS): $[MH]^+$ = 511; $[M+Na]^+$ = 533; $[M-1]^-$ = 509.

Elemental analysis: calculated: C 65.88, H 6.67, N 5.49; found C 66.00, H 6.68, N 5.47.

1H NMR ($CDCl_3$) δ = 0.90-0.95 (t, 3H, CH_3), 1.38 (s, 9H, t-Bu), 1.93-2.08 (m, 2H, CH_2), 2.8-(2.86-3.08)3.14 (dd, 2H, CH_2), 3.4 (s, 6H, CH_3), 5.06-(5.01-5.13)-5.17 (d, 2H, CH_2), 5.47 (s, 2H, CH_2), 6.24 (s, 1H, CH), 7.47 (s, 1H, CH), 7.64-7.69 (t, 1H, CH_{Ar}), 7.79-7.84 (t, 1H, CH_{Ar}), 8.23-8.32 (m, 2H, CH_{Ar}).

^{13}C NMR ($CDCl_3$) δ = 8.4; 28.2; 34.8; 45.5; 52.0; 53.0; 53.1; 59.1; 82.7; 100.6; 101.0; 124.8; 125.9; 128.0; 128.2; 130.0; 130.4; 130.5; 138.6; 142.4; 148.9; 152.9; 155.2; 162.6; 172.6.

Preparation 5

Synthesis of intermediate product 5a

383 mg (0.75 mmol) of **4a** and 564 mg (1.5 mmol, 2 eq.) of PDC were suspended in 4 ml of anhydrous CH_2Cl_2 ; the mixture thus obtained was placed under stirring at room temperature. After 16 h the solvent was removed by evaporation and the crude product thus obtained was purified by chromatography on a silica column (eluents: CH_2Cl_2 /MeOH 99/1) yielding 280 mg (0.55 mmol, 74%) of product.

$C_{28}H_{30}N_2O_7$ (506.5); m.p. (decomp.) = 210°C;

R_f = 0.64 (CH_2Cl_2 /MeOH 95/5)

MS (IS): $[M+Na]^+$ = 529; $[M-1]^-$ = 505.

Elemental analysis: calculated: C 66.40, H 5.93, N 5.53; found C 66.42, H 5.96, N 5.53.

1H NMR ($CDCl_3$) δ = 0.83-0.90 (t, 3H, CH_3), 1.33 (s, 9H, CH_3), 1.97-(2.06-2.15) 2.24 (double multiplet, 2H, CH_2), 2.90-(2.95-3.00) 3.05 (dd,

2H, CH₂), 3.42 (s, 6H, CH₃), 5.58 (s, 2H, CH₂), 6.28 (s, 1H, CH), 7.39 (s, 1H, CH), 7.68-7.76 (t, 1H, CH_{Ar}), 7.82-7.88 (t, 1H, CH_{Ar}), 8.23-8.27 (d, 1H, CH_{Ar}), 8.33-8.37 (d, 1H, CH_{Ar}).

¹³C NMR (CDCl₃) δ = 7.6; 28.1; 31.6; 43.8; 52.5; 53.1; 53.2; 82.2; 85.0; 93.8; 100.5; 114.3; 124.9; 126.4; 129.1; 130.5; 130.9; 139.1; 149.4; 151.8; 152.4; 156.2; 167.0; 167.4; 169.9.

Example 1

{10-[(E)-(ter-butoxyimino)methyl]-3-ethyl-1,13-dioxo-11,13-dihydro-1H,3H-furo[3',4':6,7]indolizino[1,2-b]quinolin-3-yl}acetic acid (ST2196)

To a solution of 71 mg (0.14 mmol) of 5a in 1.4 ml of CH₃COOH were added 44 mg (0.35 mmol, 2.5 eq.) of tBuONH₂HCl; the mixture thus obtained was stirred at 80°C and sheltered from the light for 16 h. The CH₃COOH was then removed by evaporation. The crude product thus obtained, kept sheltered from the light, was purified by chromatography on a silica column (eluent gradient: CH₂Cl₂ → CH₂Cl₂/MeOH 85/15). 45 mg (0.09 mmol, 68%) of product were obtained.

C₂₆H₂₅N₃O₆ (475.5); m.p. (decomp.) = 228°C;

R_f = 0.3 (CH₂Cl₂/MeOH 9/1).

MS (IS): [MH]⁺ = 476; [M+Na]⁺ = 498; [M-1]⁻ = 474.

Elemental analysis: calculated: C 65.68, H 5.26, N 8.84; found: C 65.70, H 5.29, N 8.83.

¹H NMR (DMSO-d₆) δ = 0.63-0.7 (t, 3H, CH₃), 1.5 (s, 9H, tBu), 2.07-2.17 (m, 2H, CH₂), 2.97-(3.03-3.23) 3.29 (dd, 2H, CH₂), 5.36 (s, 2H, CH₂), 7.64 (s, 1H, CH), 7.73-7.79 (t, 1H, CH_{Ar}), 7.89-7.96 (t, 1H, CH_{Ar}), 8.16-8.20 (d, 1H, CH_{Ar}), 8.60-8.63 (d, 1H, CH_{Ar}), 9.30 (s, 1H, CH_{Ar}).

¹³C NMR (CDCl₃) δ = 7.6; 27.8; 29.9; 31.2; 42.6; 53.1; 81.9; 85.2; 94.2; 114.2; 123.2; 125.8; 127.1; 129.0; 130.8; 130.9; 132.8; 142.2; 149.8; 151.7; 152.7; 156.2; 167.2; 170.1.

Example 2

(10-{(E)-[(benzyloxy)imino]methyl}-3-ethyl-1,13-dioxo-11,13-dihydro-1H,3H-furo[3',4':6,7]indolizino[1,2-b]quinolin-3-yl)-acetic acid (ST2285)

To a solution of 102 mg (0.2 mmol) of 5a in 2 ml of CH₃COOH were added 80 mg (0.5 mmol, 2.5 eq.) of PhCH₂ONH₂·HCl; the solution was stirred at 80°C and sheltered from the light for 16 h. The CH₃COOH was then removed by evaporation. The crude product thus obtained, kept sheltered from the light, was purified by chromatography on a silica column (eluent gradient: CH₂Cl₂ → CH₂Cl₂/MeOH 8/2). 62 mg (0.12 mmol, 61%) of product were obtained.

C₂₉H₂₃N₃O₆ (509.5); m.p. (decomp.) = 188°C;

R_f = 0.53 (CH₂Cl₂/MeOH 9/1).

MS (IS): [M+Na]⁺ = 532; [M-1]⁻ = 508.

Elemental analysis: calculated: C 68.37, H 4.52, N 8.25; found: C 68.41, H 4.50, N 8.27.

¹H NMR (DMSO-d₆) δ = 0.64-0.70 (t, 3H, CH₃), 2.05-2.17 (m, 2H, CH₂), 2.84-(2.90-3.12) 3.18 (dd, 2H, CH₂), 5.2 (s, 2H, CH₂), 5.4 (s, 2H, CH₂), 7.36-7.58 (m, 5H, CH_{Ar}), 7.62 (s, 1H, CH), 7.72-7.78 (t, 1H, CH_{Ar}), 7.85-7.90 (t, 1H, CH_{Ar}), 8.12-8.16 (d, 1H, CH_{Ar}), 8.54-8.58 (d, 1H, CH_{Ar}), 9.32 (s, 1H, CH_{Ar}).

¹³C NMR (CDCl₃) δ = 7.0; 30.6; 42.1; 52.6; 77.8; 84.8; 93.6; 113.5; 122.6; 125.2; 126.7; 128.2; 128.5; 128.6; 128.9; 130.2; 130.3; 131.2; 136.0; 143.2; 149.1; 151.0; 152.0; 155.6; 166.9; 169.7; 170.1.

Example 3

R,S-7-(1-t-butoxy)iminomethyl-homocamptothecin (ST2127);

To a solution of 510 mg (1 mmol) of 4a in 10 ml of CH₃COOH were added 314 mg (2.5 mmol, 2.5 eq) of tBuO-NH₂·HCl; the solution was maintained sheltered from the light at 80°C for 16 h. The CH₃COOH was then removed by evaporation. The crude product thus obtained,

dissolved in CH_2Cl_2 and kept sheltered from the light, was washed with water. The organic phase was dried on Na_2SO_4 , the solvent was evaporated and the crude product purified by chromatography on a silica column, keeping it sheltered from the light (eluent: $\text{CH}_2\text{Cl}_2/\text{dioxane}$ 9/1). 160 mg (0.35 mmol, 34%) of solid yellow product were obtained.

$\text{C}_{26}\text{H}_{27}\text{N}_3\text{O}_5$ (461,5); m.p. (decomp.) = 284°C ;

$R_f = 0.4$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95/5).

MS (IS): $[\text{MH}]^+ = 462$; $[\text{M}+\text{Na}]^+ = 484$; $[\text{M}-1]^- = 460$.

Elemental analysis: calculated: C 67.68, H 5.86, N 9.11; found: C 67.65, H 5.88, N 9.13.

^1H NMR (CDCl_3) $\delta = 0.87\text{--}1.03$ (t, 3H, CH_3), 1.55 (s, 9H, CH_3), 1.7-1.9 (broad, 1H, OH), 1.92-2.1 (m, 2H, CH_2), 3.26-(3.32-3.38) 3.44 (dd, 2H, CH_2), 5.13-(5.21-5.36) 5.44 (dd, 2H, CH_2), 5.35-(5.41-5.62) 5.68 (dd, 2H, CH_2), 7.43-7.50 (m, 2H, CH + CH_{Ar}), 7.60-7.65 (t, 1H, CH_{Ar}), 7.88-7.95 (t, 2H, CH), 8.86 (s, 1H, CH_{Ar}).

^{13}C NMR (CDCl_3) $\delta = 8.3$; 27.9; 35.8; 42.7; 53.4; 62.4; 73.9; 82; 101.1; 122.9; 123.4; 125.1; 125.5; 128.3; 130.1; 130.4; 132.5; 142.1; 144.7; 149.0; 151.6; 156.4; 160.0; 171.6.

HPLC analysis of (R,S) ST 2127 on a chiral column using a circular dichroism detector revealed the separation of the two enantiomers.

The enantiomers of (R,S) ST 2127 were isolated via HPLC by preparative chromatography on a chiral column in the following conditions:

column : (S,S)- DACH-DNB 5/100;

eluent: $\text{CH}_2\text{Cl}_2/\text{n-HEXANE}$ (80/20) + 1% MeOH;

flow rate: 1 ml/min;

T: 22°C ;

λ 360 nm UV detector.

The first eluted fraction with e.e. 99.46% corresponds to (+) ST2127 = ST2522 to which the R configuration was attributed by analogy with

the camptothecins with positive $[\alpha]_D$.

$IC_{50} = 18 \text{ nM} \pm 0.2$ (H460).

The second eluted fraction with e.e. 99.46% corresponds to (-) ST2127 = ST2523 with $[\alpha]_D = -48.11 \pm 0.15$ ($c = 1.17$; $CHCl_3$ - MeOH 4:1).

The S configuration was attributed to A ST2523 by analogy with the camptothecins with negative $[\alpha]_D$.

$IC_{50} = > 200 \text{ nM}$ (H460).

Example 4

R,S-7-benzyloxyiminomethyl-homocamptothecin (ST2143):

To a solution of 510 mg (1 mmol) of 4a in 10 ml of CH_3COOH were added 400 mg (2.5 mmol) of $PhCH_2ONH_2 \cdot HCl$; the solution was kept sheltered from the light and stirred at $80^\circ C$ for 16 h. The CH_3COOH was then removed by evaporation. The crude product thus obtained was purified by chromatography on a silica column (eluent: CH_2Cl_2 /dioxane 9/1). 223 mg of product were obtained as a yellow solid (0.45 mmol, yield 45%).

$C_{29}H_{25}N_3O_5$ (495.5); m.p. (decomp.) = $263^\circ C$;

$R_f = 0.48$ (CH_2Cl_2 /MeOH 95/5).

MS (IS): $[MH]^+ = 496$; $[M+Na]^+ = 518$; $[M-1]^- = 494$.

Elemental analysis: calculated: C 70.30, H 5.05, N 8.48; found: C 70.33, H 5.09, N 8.47.

1H NMR ($CDCl_3$) $\delta = 0.95$ -1.02 (t, 3H, CH_3), 1.99-2.06 (m, 2H, CH_2), 3.04-(3.08-3.18)3.42 (dd, 2H, CH_2), 5.32 (s, 2H, CH_2), 5.42-(5.44-5.63) 5.70 (dd + s, 4H, CH_2 + CH_2), 7.33-7.56 (m, 5H, CH_{Ar}), 7.63-7.69 (m, 2H, CH + CH_{Ar}), 7.80-7.84 (t, 1H, CH_{Ar}), 8.16-8.22 (m, 2H, CH_{Ar}), 9.10 (s, 1H, CH).

^{13}C NMR ($CDCl_3$) $\delta = 8.4$; 36.5; 42.6; 52.9; 62.2; 73.6; 78.1; 101.1; 123.0; 123.1; 125.3; 126.2; 128.3; 128.5; 128.8; 129.1; 130.3; 130.4; 131.4;

136.5; 144.0; 144.4; 149.2; 152.5; 156.4; 159.7; 172.1.

Example 5

Ter-butyl ester of (3-ethyl-1,13-dioxo-11,13-dihydro-1H,3H-furo[3',4':6,7]indolizino[1,2-b]quinolin-3-yl)acetic acid (ST2084)

To a solution of 1 g (2.3 mmol) of 4b in 10 ml of anhydrous CH_2Cl_2 were added 1.73 g (4.6 mmol, 2 eq.) of PDC. The mixture was stirred at room temperature for 16 h. At the end of this period the reaction was brought to dryness and purified by flash chromatography on a silica column (eluent: $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95/5). 726 mg (1.68 mmol, 73%) of product were obtained as a yellow solid.

$\text{C}_{25}\text{H}_{24}\text{N}_2\text{O}_5$ (432.5); m.p. (decomp.) = 190°C ;

$R_f = 0.5$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95/5).

MS (IS): $[\text{MH}]^+ = 432$; $[\text{M}+\text{Na}]^+ = 455$.

Elemental analysis: calculated: C 69.44, H 5.56, N 6.48; found: C 69.46, H 5.55, N 6.51.

^1H NMR (CDCl_3) δ = 0.83-0.88 (t, 3H, CH_3), 1.35 (s, 9H, t-Bu), 1.95-2.27 (m(double multiplet), 2H, CH_2), 2.91-(2.96-3.01) 3.06 (dd, 2H, CH_2), 5.38 (s, 2H, CH_2), 7.36 (s, 1H, CH), 7.68-7.75 (t, 1H, CH_{Ar}), 7.83-7.90 (t, 1H, CH_{Ar}), 7.97-8.00 (d, 1H, CH_{Ar}), 8.22-8.25 (d, 1H, CH_{Ar}), 8.46 (s, 1H, CH_{Ar}).

^{13}C NMR (CDCl_3) δ = 7.5; 28.1; 31.6; 43.7; 50.6; 82.2; 85.2; 94.0; 114.3; 128.5; 128.9; 130.1; 131.2; 131.7; 149.3; 151.8; 153.0; 167.4; 170.2.

Example 6

(3-ethyl-1,13-dioxo-11,13-dihydro-1H,3H-furo[3',4':6,7]indolizino[1,2-b]quinolin-3-yl)acetic acid (ST2085)

110 mg (0.25 mmol) of EM 21/2 were dissolved in 1.5 ml of a 1:1 mixture of $\text{CH}_2\text{Cl}_2/\text{TFA}$. The mixture was stirred at room temperature for 16 h. The solvent was then evaporated dry to yield 94 mg of product

as a yellow solid (0.25 mmol, quantitative yield).

$C_{21}H_{16}N_2O_5$ (376.4); m.p. (decomp.) = 242°C;

R_f = 0.25 (CH_2Cl_2 /MeOH 95/5).

MS (IS): $[MH]^+ = 377$; $[M+Na]^+ = 399$; $[M-1]^- = 375$.

Elemental analysis: calculated: C 67.02, H 4.26, N 7.45; found C 67.05, H 4.28, N 7.49.

1H NMR (DMSO- d_6) δ = 0.64-0.70 (t, 3H, CH_3), 2.03-2.16 (m, 2H, CH_2), 3.05-(3.10-3.30) 3.35 (dd, 2H, CH_2), 4.00-4.75 (broad, 1H, OH), 5.33 (s, 2H, CH_2), 7.65 (s, 1H, CH), 7.73-7.78 (t, 1H, CH_{Ar}), 7.84-7.90 (t, 1H, CH_{Ar}), 8.15-8.18 (d, 2H, CH_{Ar}), 8.73 (d, 1H, CH_{Ar}).

^{13}C NMR ($CDCl_3$) δ = 7.7; 31.2; 41.6; 51.5; 85.3; 94.5; 113.5; 129.0; 129.1; 129.4; 129.8; 131.4; 131.9; 132.6; 148.8; 152.5; 153.5; 156.0; 167.4; 170.5; 170.6.